Florida Citrus Advanced Technology Program

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Psyllid	1/15/10

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Psyllid gut genes cloned, TMV-based anti- psyllid cDNA library is developed.

TITLE and CONTACT INFORMATION

Proposal Title Controlling HLB by controlling psyllids with RNA interference

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Organization University of California, Davis	*Total Direct Funds (current year)* $198,274

REPORT UPDATE (650 words; provide details about your headline)

We are in the first year of our project. Our goal is to identify and then use specific psyllid RNA sequences to induce RNA interference (RNAi) activity in recipient psyllids. We propose to express psyllid interfering RNA sequences in plants and induce RNAi when psyllids feed on these plants. We envision that successful application of RNAi towards the Asian citrus psyllid (ACP, D. citri) will confer a negative phenotype (even death) in psyllids, such that psyllids cannot colonize and/or reproduce on selected plants. By controlling the psyllid vector we believe this will complement other efforts to help control HLB. To make rapid progress we are using the tomato psyllid (Bactericerca cockerelli), which colonizes herbaceous plants (tomatoes, tobaccos) and is the vector of another Liberibacter spp. (C. L. psyllaurous). We are using tomatoes (Early Pak 7), and Turkish tobacco plants for evaluating Tobacco mosaic virus (TMV)-driven expression of psyllid genes for inducing RNAi. Both of these plants are easy to use, TMV susceptible and both are readily colonized by B. cockerelli. With Turkish tobacco we obtain more consistent Agrobacterium tumefaciens-based TMV infection than for tomatoes, but both plants, as well as Nicotiana benthamiana, are being used currently, although psyllids do not prefer N. benthamiana. We can induce production of specific siRNAs corresponding to the recombinant sequences in these plants (N. benthamiana and tomato), and we have demonstrated so far that psyllids can acquire TMV-based RNAs from TMV-infected tomato plants. Now we are evaluating acquisition and effects of anti-psyllid RNAs.

We have utilized two approaches to obtain psyllid interfering RNAs for our work. The first has been by mining existing GenBank data for potentially useful D. citri sequences, and the second has been by constructing a new, normalized cDNA library for B. cockerelli mRNAs directly in a TMV-based virus delivery system. Both approaches are proceeding very well. We have used the D. citri EST database and have identified 1904 contigs, 179 of which are predicted to be expressed in D. citri midguts. We believe that midgut sequences are very likely good targets for orally-acquired anti-psyllid interfering RNAs or dsRNAs, as the midgut will encounter RNAs via psyllids feeding on plants. We have now successfully cloned 80 midgut genes (partial sequences varying in size between 500-1000bp) from D. citri. BLAST comparative sequence analyses allowed us to design 56 predicted conserved primers for use in cloning D. citri homologs from B. cockerelli. We have cloned 36 putative homologs so far, and 30 have been confirmed by sequence analysis. These are highly homologous (80 – 90%) to corresponding D.citri sequences. So far RT-PCR using RNA prepared from B. cockerelli gut tissues confirmed that 25 were present. Sequence-based analyses identified some, including ion transporter genes, which are ideal candidates for RNA interference, and we are proceeding using TMV-based expression as well as direct micro injection to determine their efficacy against B. cockerelli. We have developed a micro-injection system for psyllids. This allows precise injection of known quantities of dsRNAs into the psyllid hemocoel. This is being used as a known control approach to assist in identifying candidate sequences for RNAi. Sequences that show effects after injection will be immediately tested in plants.

We now have a normalized B. cockerelli cDNA library. The library sequences are cloned directly into our TMV-based plasmid for expression in plants. We are now confirming the library quality by nucleotide sequence analysis, and transforming the TMV-based plasmids into A. tumefaciens for use in whole plant anti-psyllid assays. Initial sequencing showed us that the library contains psyllid sequences and thus will be very useful for our effort.